

A General Theory for Direct Quantitative Analysis of Antigen

Feng Gan¹*, Gang Ye², Langxia Liu³, Xiaohui Liu³

1. School of Chemistry and Chemical Engineering, Sun Yat-Sen University,
Guangzhou 510275, P.R. China

2. Guangzhou Overseas Chinese Hospital, Guangzhou 510630, China

3. College of Life Science and Technology, Jinan University, Guangzhou 510630, China

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Abstract

A theory for direct quantitative analysis of an antigen is proposed. It is based on a potential homogenous immunoreaction system. It establishes an equation to describe the concentration change of the antigen and antibody complex. A maximum point is found in the concentration profile of the complex which can be used to calculate the concentration of the antigen. An experimental scheme was designed for a commercial time-resolved fluoroimmunoassay kit for HBsAg, which is based heterogeneous immunoreaction. The results showed that the theory is practically applicable.

1 Introduction

Antigen is a substance that was introduced into the body of a living organism, which will lead to a disease sometimes. The invading of antigen will trigger the generation of an substance called antibody by the immune system of the organism. The antibody plays the role to kill or neutralize the antigen. This kind of reaction between antigen and antibody have found application in qualitative analysis and quantitative analysis of antigen. The main topic of this paper focuses on the quantitative analysis.

A well known method to detect an antigen is the enzyme-linked immunosorbent assay (ELISA)[1, 2, 3]. It mostly focuses on detecting the presence of an antigen. With the increasing demand on quantitative analysis of antigen in the diagnosis and treatment of a disease such as hepatitis B, the time-resolved fluoroimmunoassay (TRFIA) gradually shows its advantages[4, 5, 6]. The TRFIA makes the best use of fluorescence delay effect of europium (Eu) to eliminates the background fluorescence[7]. So, the TRFIA is especially adapted in complicated sample such as sera.

There is a problem with the existing quantitative methods for antigens. They usually need the standard substance of the antigen in the calibration procedure of a measurement[8]. This procedure has been an indispensable part of presently existing quantitative analysis based on analytical instruments.

*Corresponding author, Email: cesgf@mail.sysu.edu.cn

However, the expense on the standard substance is a huge burden when the substance is expensive. Furthermore, when the standard substance is not available, the quantitative analysis can not be implemented.

The purpose of this paper is to establish a general theory of quantitative analysis that does not rely on the standard substance of target antigen. The theory was developed based on a potential homogenous immunoreaction system. It was finally applied to the direct quantitative analysis of HBsAg by designing an experimental scheme for heterogeneous immunoreaction.

2 Theory

Figure 1 shows the fundamental principal and experimental steps of a commercial time-resolved fluoroimmunoassay (TRFIA) kit for HBsAg. The double antibodies sandwich technology ensures that one antigen (Ag) combines with one labeled antibody (Abx) [9]. This kind of reaction mode is the requirement for quantitative analysis of the Ag. Thus, the reaction between Ag and Abx can be expressed as follows:



where x represents a label such as Eu^{3+} . Final measurement is usually based on the label. We assume the initial concentrations of Ag and Abx are C_{Ag} and C_{Abx} , respectively. When V_t volume of Abx's solution is added into V_0 volume of Ag's solution, the mass balances are as following

$$\frac{C_{\text{Ag}}V_t}{V_0 + V_t} = [C_{\text{Ag}}] + [\text{Ag} \cdot \text{Abx}] \quad (2)$$

$$\frac{C_{\text{Abx}}V_t}{V_0 + V_t} = [C_{\text{Abx}}] + [\text{Ag} \cdot \text{Abx}] \quad (3)$$

where $[]$ represents the equilibrium concentration of the corresponding species. From equation (2) and equation (3), we get

$$[\text{Ag} \cdot \text{Abx}] = \frac{g(\rho) - \sqrt{g^2(\rho) - h(\rho)}}{2} \quad (4)$$

$$\text{where } \rho = \frac{V_t}{V_0}; g(\rho) = \frac{\rho C_{\text{Abx}} + C_{\text{Ag}}}{1 + \rho} + \frac{1}{K}; h(\rho) = \frac{4\rho C_{\text{Ag}} C_{\text{Abx}}}{(1 + \rho)^2}; K = \frac{[C_{\text{Ag}} \cdot \text{Abx}]}{[C_{\text{Ag}}][C_{\text{Abx}}]}.$$

There is a maximum point in the plot of $[\text{Ag} \cdot \text{Abx}]$ versus ρ according to equation (4). The first derivation of $[\text{Ag} \cdot \text{Abx}]$ versus ρ is

$$[\text{Ag} \cdot \text{Abx}]' = \frac{g'(\rho)}{2} - \frac{1}{4}[g^2(\rho) - h(\rho)]^{-\frac{1}{2}}[2g(\rho)g'(\rho) - h'(\rho)] \quad (5)$$

$$\text{where } g'(\rho) = \frac{C_{\text{Abx}} - C_{\text{Ag}}}{(1 + \rho)^2}; h'(\rho) = \frac{4C_{\text{Ag}} C_{\text{Abx}}(1 - \rho)}{(\rho + 1)^3}.$$

Let $[\text{Ag} \cdot \text{Abx}]' = 0$, we finally get

$$\rho_0 = \frac{C_{\text{Ag}} C_{\text{Abx}} \pm \sqrt{\left(C_{\text{Ag}} + C_{\text{Abx}} + \frac{1}{K}\right) \frac{(C_{\text{Ag}} - C_{\text{Abx}})^2}{K}}}{C_{\text{Abx}}^2 + \frac{C_{\text{Abx}} - C_{\text{Ag}}}{K}} \quad (6)$$

where ρ_0 is the ρ value at the maximum point.

And it can be proved that the ρ_0 at the maximum point will be

$$\lim_{K \rightarrow \infty} \rho_0 = \frac{C_{Ag}}{C_{Abx}}. \quad (7)$$

Equation (7) shows that the concentration of the Ag can be calculated without using calibration procedure. The requisite condition is that the generated antigen antibody complex, the $Ag \cdot Abx$, is stable enough. The relative error of the estimation is

$$r.e. = \frac{\rho_0 - \frac{C_{Ag}}{C_{Abx}}}{\frac{C_{Ag}}{C_{Abx}}} = \frac{C_{Abx}^2 \pm \frac{C_{Abx}}{C_{Ag}} \sqrt{\left(C_{Ag} + C_{Abx} + \frac{1}{K}\right) \frac{(C_{Ag} - C_{Abx})^2}{K}}}{C_{Abx}^2 + \frac{C_{Abx} - C_{Ag}}{K}} - 1. \quad (8)$$

Obviously, $\lim_{K \rightarrow \infty} r.e. = 0$. Similar results can be obtained when $C_{Ag} = C_{Abx}$.

Above theory is actually based on homogeneous system. The final measurement is based on labeled antibody. It needs quite an effort to apply the theory to practical system because of the lack of proper reagent kit. This situation could be changed in near future by using the homogeneous fluoroimmunoassay (HFIA)[9]. However, a properly designed experimental scheme can apply the theory in another way.

3 An application to TRFIA of HBsAg

Figure 1 shows a standard scheme for quantitative analysis of HBsAg using TRFIA. In the first incubation, the added HBsAg is fixed by the solid phase anti-HBs in the well. So, in the second incubation, the introduced Eu-labeled anti-HBs will combine with the HBsAg in 1:1 ratio. The measured fluorescence intensity of Eu in final step will be directly propotional to the quantity of the HBsAg.

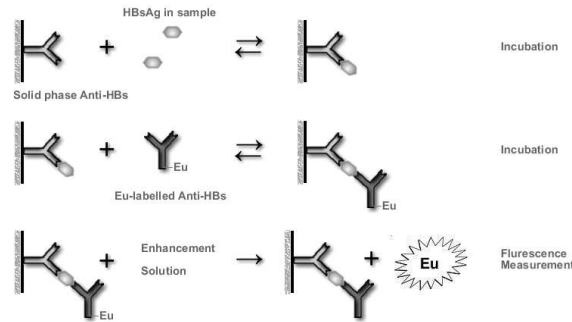


Figure 1: The basic steps of a commercial TRFIA kit for HBsAg.

We designed an experimental scheme and shown it in Table 1. In the scheme, the quantities of HBsAg in the wells are fixed which limits the binding quantity of the Eu-labelled anti-HBs in each of the wells. For the convenience of discussion, we assume the HBsAg quantity in each well is $C_{HBsAg}V_0$ mol. When $C_{anti-HBs-Eu}V_t > C_{HBsAg}V_0$, extra added Eu-labelled anti-HBs will be washed out. On the other hand, when $C_{anti-HBs-Eu}V_t < C_{HBsAg}V_0$, the added Eu-labelled anti-HBs is totally combined with the HBsAg. The equations (2) and (3) hold under the latter situation.

Table 1: An experimental scheme for direct quantitative analysis of HBsAg

Wells	1	2	3	4	5	6	7	8
V_0 (μL) ^a	100	100	100	100	100	100	100	100
V_t (μL) ^b	20	40	60	80	100	120	140	160
$(V_0 + V_t)$ (μL) ^c	120	140	160	180	200	220	240	260

^a Volume of HBsAg.

^b Volume of anti-HBs-Eu.

^c Volume of enhancement solution.

The more important thing is that the final measurement is based on the quantity of Eu released by enhancement solution rather than antigen or antibody or antigen antibody complex. So, if we put the Eu in a volume variation environment like equations (2) and (3), equation (4) can be used to describe its concentration change. Finally, equation (7) can be used to calculate the concentration of HBsAg. The third line in Table 1 simulates equation (3) by adding Eu-labelled anti-HBs into the wells progressively. The most important operation is listed in the last line of Table 1, which offers the final Eu a circumstance of volume variation. So, Eu has same concentration change as the antigen and antibody complex as shown in equation (4).

4 Experiments

The TRFIA kit for HBsAg were from Guangzhou Darui Antibody Engineering And Technology Co. Ltd.. The sera for quality control were taken as unknown samples. The instrument was the Victor X5 Multilabel Plate Reader of PerkinElmer. TopPette Pipettors (Dragon Medical (Shanghai) Ltd.) were used to add solutions. The volumes of the reagents are shown in Table 1. Experimental operations were done in accordance with the kit's manual. Five repeat measurements were implemented.

5 Results

Figure 2 shows the fluorescence intensities of Eu versus volume ratio. The solid line is the curve fitting result of the mean intensities using equation (4). The maximum point in the curve is used to calculate the concentration of the HBsAg. Table 2 shows the calculated results of two sera. It must note that fluorescence intensity should be adjust to unit volume because the instrument only recordes total fluorescence intensity.

6 Conclusion

We successfully established a general theory for the direct quantitative analysis of antigen. The theory is self-contained mathematically. The application examples also show practicability of the theory. The

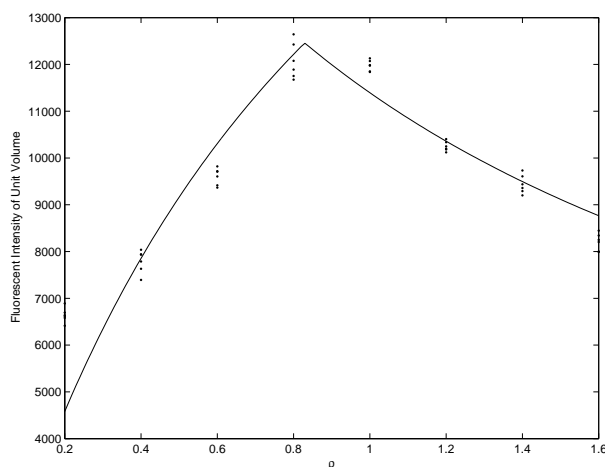


Figure 2: An example of fluorescence intensity of Eu with ρ .

Table 2: Measurement results of HBsAg ($C_{\text{anti-HBs-Eu}} = 0.00627 \mu\text{mol/L}$)

Real concentration of HBsAg	Mean ρ value at max point, variance and repeat number	Calculated concentration of HBsAg	con- of error
0.00417 $\mu\text{mol/L}$	0.760, 0.035, 5	0.00477 $\mu\text{mol/L}$	14.3%
0.00625 $\mu\text{mol/L}$	0.813, 0.0050, 5	0.00510 $\mu\text{mol/L}$	-18.5%

advantage of the theory can be shown when the standard substances are extremely expensive or there are even no standard substances available. More experiments are still needed to make it a routine method.

Acknowledgments

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